

GAPS II Coated Slides

Instruction Manual



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For Research Laboratory Use Only

Cat. No. 40003 – Slides with Bar Code

Cat. No. 40005 – Slides with Bar Code (bulk packaged)

Cat. No. 40004 – Slides without Bar Code

Cat. No. 40006 – Slides without Bar Code (bulk packaged)

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CONTENTS

Introduction	1
Overview	1
Handling and Care Instructions	1
Storage Instructions	2
DNA Array Preparation and Hybridization	
Protocols	2
General Considerations	2
Printing DNA arrays on GAPS II slides	4
References	9
Corning Products for Printing Microarrays	10

INTRODUCTION

Overview

Corning® GAPS II Coated slides have a uniform, covalently bound coating of pure Gamma Amino Propyl Silane that gives the slides a high quality, DNA immobilizing surface.

The quality of nucleic acid arrays produced is highly dependent on the substrate. A poor quality coated glass slide will lead to problems with spot uniformity and morphology as well as high and varying background fluorescence. The spots may vary in size, shape, and DNA retention due to varying surface energies across the slide. Scratches and foreign material on the slide surface also cause deformation of the array as well as varying background fluorescence. These quality issues all lead to a loss in sensitivity and generally poor results.

GAPS II slides are manufactured under the most stringent conditions to overcome these quality issues. All slides are cleaned and individually examined for mechanical defects and the presence of dust and glass particles. Using a proprietary process, GAPS is applied in an environmentally controlled, HEPA-filtered ISO Class 5 facility, resulting in coated slides with highly uniform surface properties and low autofluorescence. Surface wettability is consistent across the slide surface to assure uniform spot size and shape and to avoid uncontrolled wicking or poor volume transfer during the print. Amine density is also uniform across the slide surface leading to uniform DNA retention across the printed array. Packaging has been developed to maintain the appropriate storage environment.

Handling and Care Instructions

GAPS II slides are manufactured by a carefully controlled manufacturing process to maximize their performance. To assure this performance, please follow these recommendations:

- ▶ Use the slides in a clean environment. Particles falling onto the slide surface may cause defects in the printed array as well as nuclease contamination. Self-contained printing environments may be required to prevent such contamination.

- ▶ Avoid direct contact with the surface of the slide to be printed. Only the print pins and processing solutions should touch the print area to avoid contamination and abrasion of the coating. The coating is very thin and contact with the surface can affect its integrity.
- ▶ When using slides without bar codes, always print on the side facing away from the wall of the plastic container. Clearly mark the side to be printed using a glass-etching tool.
- ▶ If the package has been inadvertently stored at temperatures lower than 20°C, allow the foil pouch to come to room temperature before opening. Otherwise, condensation may form on the slide surface, negatively affecting the wettability of the coating.
- ▶ Open the metalized foil pouch just prior to printing. Close the cap on the slide container as soon as possible after removing slides for use to maintain a closed environment for unused slides. Place the closed container in the foil pouch to protect the remaining slides and store them in a desiccator. Use the remaining slides within one week.
- ▶ The original slide container protects the print zone on the slide's surface. The container is therefore a good place to store slides after printing and/or hybridizing. Keep arrays hybridized with fluorescent probes in a light-tight box to prevent photobleaching.

Storage Instructions

For best results, store product at room temperature (20 to 25°C) in original undamaged packaging, and use slides by the date indicated on the label. After opening, store as described in the Handling and Care Instructions.

DNA ARRAY PREPARATION AND HYBRIDIZATION PROTOCOLS

General Considerations

The surface of GAPS II Coated slides is highly reactive towards DNA. The key to producing microarrays of high quality is to take advantage of this high reactivity during the

printing process while minimizing the spurious attachment of nucleic acids to the unprinted area during subsequent manipulation of the array. The following are some of the most critical factors to consider:

- ▶ *Concentration of the DNA.* The high reactivity of GAPS II Coated slides allows the use of printing solutions containing as little as 25 ng of DNA per microliter. The optimal concentration needs to be determined empirically. We recommend 0.1 mg/mL as a starting point. When too little DNA is used, the printed spots will not reach signal saturation levels, thus reducing the dynamic range of the array; on the other hand, highly concentrated printing solutions can produce spots with “comet tails” and other forms of localized background. The concentration and purity of the DNA should be checked spectrophotometrically as well as electrophoretically.
- ▶ *Composition of the printing solution.* The chemical and physical properties of the solvent greatly influence DNA retention, spot morphology, and hybridization efficiency. The ideal printing solution is one that denatures the DNA and has low evaporation rate. Denatured DNA binds more efficiently to the GAPS coating and readily hybridizes to the labeled probe. Ionic attachment of the negatively charged phosphate groups of the DNA backbone to the free amine groups on the surface of the slide occurs in the liquid phase. Solvent evaporation causes the concentration of DNA and other nonvolatile components of the printing solution to rise, leading to time-dependent changes in spot quality and the eventual loss of the printing solution. A solvent that evaporates slowly increases DNA retention and spot uniformity.
- ▶ *Immobilization procedures.* Binding of DNA to the GAPS coated surface is enhanced by UV crosslinking and baking. These procedures work equally well at immobilizing the printed DNA.
- ▶ *Blocking procedures.* Although most forms of background fluorescence are not additive to the signal intensity of printed spots, their occurrence is esthetically unpleasant and may interfere with spot identification during image analysis. Deactivating the unused surface of the slide greatly reduces the incidence of high background. For this purpose, we

strongly recommend that albumin be included in the prehybridization buffer, as described below.

- ▶ *Arrayer settings and pin quality:* Follow the instructions provided by the manufacturer of arraying equipment and printing pins. Pin contact time and the force with which the pin strikes the slide affect spot size and morphology. It is strongly recommended that the printing pins be qualified before use. Pins that are either broken or out-of-spec can ruin otherwise good arrays.

The quality of the probe and the hybridization and washing conditions greatly influence the performance of printed arrays. Optimized protocols for the use of Corning® GAPS Coated Slides have been published^{1,2} and should be used as starting points to find the printing and processing conditions that best fit the reality of each laboratory. The following protocols have been successfully used in Corning laboratories.

Printing and Hybridization of DNA Arrays on GAPS II Slides

Printing Solution Selection

Various formulations have been used for printing DNA arrays on GAPS slides. Final determination as to what solution to use is left for the user to make. Do not add detergents to the printing solution, as their presence inhibits binding between the DNA and the GAPS molecules.

3X SSC, 1.5 M betaine²

Advantages

- ▶ Denatures the DNA
- ▶ Low evaporation rate
- ▶ Interacts well with GAPS coating, producing more uniform spots

Disadvantages

- ▶ Very hygroscopic; best when arrays are processed immediately after baking

50% DMSO

Advantages

- ▶ Denatures the DNA
- ▶ Low evaporation rate
- ▶ Interacts well with GAPS coating, producing more uniform spots

Disadvantages

- ▶ Strong irritant
- ▶ Produces spots of large diameter, sometimes causing spots to merge
- ▶ Causes aggregation of DNA at concentrations of DMSO higher than 70%

3X SSC

Advantages

- ▶ Commonly used aqueous solvent
- ▶ Produces spots of small diameter, allowing high printing density

Disadvantages

- ▶ Does not denature the DNA
- ▶ High evaporation rate, requiring carefully controlled printing environment

150 mM NaPO₄, pH 8.5

Similar to 3X SSC in terms of advantages and disadvantages

Array Printing

As the GAPS surface provides free amine groups for ionic attachment of the negatively charged phosphate groups of the DNA backbone, the DNA to be printed need not be derivatized with an amine or other chemical group. Such modifications, however, will not interfere with the interaction.

1. Resuspend DNA to a maximum of 0.25 mg/mL (0.1 mg/mL is a good starting concentration for optimization) in your choice of printing solution.

2. Transfer resuspended DNA to Corning plates (Cat. No. 3656 for 384 well or Cat. No. 3357 for 96 well).
3. Setup arrayer and print slides (bar code label side up) according to manufacturer's or laboratory protocol. Be sure to label side used for printing when using slides without bar code.

Array Stabilization and Immobilization

1. Rehydrate arrays by holding slides (array side down) over a bath of hot double distilled H₂O (95 to 100°C) for approximately 5 sec until a light vapor film is observed across the slide.
2. Snap-dry each array (DNA side up) on a 100°C hot plate for approximately 5 to 10 sec.
3. UV cross-link DNA to the slide by using a UV crosslinker (150 to 300 mJoules) or by baking the array at 80°C for 2 to 4 hours. Care should be taken regarding the cleanliness of the baking oven. Volatile organics can irreversibly contaminate the surface of the array leading to high backgrounds.

Array Hybridization

Preparation of Probe Solution

Most DNA arrays are hybridized with fluorescently labeled cDNA using various reverse-transcription protocols and commercially available reagents. The quality and cleanliness of the starting RNA and the resulting cDNA are critical factors for successful use of the arrays. It is recommended that the labeled cDNA be purified and quantitated spectrophotometrically.

Use between 20 and 45 µL of probe solution, depending on the size of the printed area and coverslip. Do not exceed a probe concentration of 10 ng/µL of cDNA. Best results are obtained with probes having a frequency of incorporation (FOI) of 20 to 50 labeled nucleotides per 1,000 nucleotides of cDNA. The FOI can be calculated using the following formulae.

$$\begin{aligned} \text{Amount of cDNA probe (ng)} = \\ A_{260} \times 37 \times \text{total volume of probe (}\mu\text{L)} \end{aligned}$$

$$\begin{aligned} \text{pmol of dye incorporated} &= \\ &\text{for Cy3}^{\text{TM}}: A550 \times \text{total volume of probe}/0.15 \\ &\text{for Cy5}^{\text{TM}}: A650 \times \text{total volume of probe}/0.25 \\ \text{FOI} &= \text{pmol of dye incorporated} \times 324.5/\text{ng of} \\ &\text{cDNA probe} \end{aligned}$$

When using more than one type of fluorescent nucleotide, such as the commonly used combination of cyanine-3 and cyanine-5 dyes, mix equivalent amounts of labeled cDNA, measured as the number of pmoles of incorporated Cy-dCTP.

Hybridization option A: Hybridization Using Formamide
Optimized probe preparation and hybridization instructions can be found on the Life Sciences web site www.corning.com/lifesciences in the technical bulletin titled: "Detailed Instructions for Hybridization of cDNA Probes to dsDNA Arrays Made on GAPS Slides."

Pre-Hybridization

1. Incubate arrays in 25 to 50% formamide, depending on the desired stringency, 5X SSC, 0.1% SDS, 0.1 mg/mL BSA in a Coplin jar for 30 to 60 minutes at 42°C.
2. Wash arrays by immersing in water followed by rinsing in isopropanol. Make sure the SDS is completely removed from the arrays during this step.
3. Dry arrays by centrifugation or blow dry using compressed N₂.

Hybridization

1. Prepare probe in fresh hybridization solution consisting of 25 to 50% formamide (use the same formamide concentration as for pre-hybridization), 5X SSC, 0.1% SDS, and 0.1 mg/mL of a nucleic-acid blocker of choice, such as sonicated herring or salmon sperm DNA, human Cot1 DNA, etc.
2. Incubate the probe solution at 95°C for 5 min.
3. Centrifuge the probe for 2 min to collect condensate and let sample cool to room temperature.

4. Place array in Corning Hybridization Chamber (Cat. No. 2551). Pipette the probe onto the surface of the printed side of the slide. Carefully place the coverslip on top of the array in such a manner as to avoid the formation of air bubbles under the coverslip. Small air bubbles that do form usually dissipate during hybridization. Assemble the chamber as described in its package insert.
5. Submerge the chamber in a 42°C water bath or place in a hybridization oven overnight.

Hybridization Option B: Hybridization Without Formamide

Pre-Hybridization

1. Incubate arrays in 3X SSC, 0.1% SDS, 0.1 mg/mL BSA in a Coplin jar for 30 to 60 min at 50°C.
2. Wash arrays by dipping in water followed by rinsing in isopropanol. Make sure the SDS is completely removed from the arrays during this step.
3. Dry arrays by centrifugation or blow-dry using compressed N₂.

Hybridization

1. Prepare probe in 3X SSC, 0.1% SDS, and 0.1 mg/mL of a nucleic-acid blocker of choice, such as sonicated herring or salmon sperm DNA, human Cot1 DNA, etc.
2. Heat at 95°C for 1 min.
3. Centrifuge the probe for 2 min to collect condensation, and let sample cool to room temperature.
4. Place array in Corning Hybridization chamber (Cat. No. 2551). Pipette the probe onto the surface of the printed side of the slide. Carefully place the coverslip on top of the slide in such a manner as to avoid the formation of air bubbles under the coverslip surface.
5. Small air bubbles that do form usually dissipate during hybridization. Assemble the chamber as described in its package insert.
6. Submerge the chamber in a 50°C water bath or place in a hybridization oven overnight.

Post-Hybridization Washing

DO NOT WASH SLIDES THAT HAVE BEEN HYBRIDIZED WITH DIFFERENT PROBES IN THE SAME WASH CONTAINERS.

DO NOT WASH MORE THAN 4 SLIDES IN A 200 ML STAINING JAR.

DO NOT ALLOW ARRAYS TO DRY OUT BETWEEN WASHES.

1. Disassemble the hybridization chamber right side up.
2. Remove the coverslip by immersing the array in 2X SSC, 0.1% SDS (at 42°C) until the coverslip moves freely away from the slide.
3. Place array in 2X SSC, 0.1% SDS for 5 min at 42°C.
4. Place array in 0.1X SSC, 0.1% SDS for 10 min at room temperature.
5. Place array in 0.1X SSC for 1 min at room temperature. Repeat 4 times.
6. Rinse array in 0.01X SSC for up to 10 sec or less.
7. Dry arrays by centrifugation or blow-dry using compressed N₂.
8. Scan.

REFERENCES

1. Hedge, P. et al, 2000. A concise guide to microarray analysis. *Biotechniques* 29:548-562.
2. Diehl, F et al, 2001. Manufacturing DNA microarrays of high spot homogeneity and reduced background signal. *Nucleic Acids Research* 29, e3 8.

For questions, further clarification about this protocol, and other technical issues and information not covered in this manual, please e-mail **actoncs@acton.corning.com** or call 800-492-1110 (978-635-2200 outside Canada and USA). A detailed hybridization protocol can be found on our website at **www.corning.com/lifesciences**.

CORNING PRODUCTS FOR MICROARRAY PRINTING

Cat. No.	Product Description	Qty/Pk	Qty/Cs
40003	GAPS II coated slides with bar code	5	25 slides
40004	GAPS II coated slides without bar code	5	25 slides
40005	GAPS II coated slides with bar code, bulk	25	25 slides
40006	GAPS II coated slides without bar code, bulk	25	25 slides
40015	UltraGAPS™ coated slides with bar code	5	25 slides
40016	UltraGAPS coated slides without bar code	5	25 slides
2551	Hybridization Chamber	1	5 chambers
40001	Hybridization Chamber O-rings	5	5 rings

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Corning has applied for patents concerning the use of GAPS coated slides in GPCR membrane microarray applications. GAPS II coated slides are not manufactured to the specifications required for use in this application. Purchase of these slides does not imply a license to use GAPS II coated slides for GPCR membrane applications.

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